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The Recognition of Diazinon, An Organophosphorus Pesticide, when Found in Samples in the Form of Decomposition Products

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The Recognition of Diazinon, an Organophosphorus Pesticide, When Found in Samples in the Form of Decomposition Products

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Abstract

The contents of a bottle, from which a human being was reported to have drunk and which were believed responsible to an organophosphorus poisoning, were submitted for chemical analysis. Initial screening by gas chromatography with phosphorus, sulfur, and nitrogen specific detectors failed to identify any intact organophosphorus pesticide. Mass spectrometric techniques were applied to the identification. Field ionization, field desorption, chemical ionization, exact mass measurements at high resolution, and GC/low resolution mass spectrometry were used to help define the qualitative and partial quantitative nature of the sample components. Results of this study were consistent with the virtually complete conversion of the pesticide, diazinon [Chemical Abstracts reference number 333-41-5], into a mixture of at least twenty-six chemically distinct products or impurities. The most abundant chemical compounds found in the sample included: 2-isopropyl-4-methyl-6-hydroxypyrimidine [2814-20-2]; 2-isopropyl-4-methyl-6-mercaptopyrimidine; 6,6′-dithiobis-(2-isopropyl-4-methylpyrimidine); 6,6′-thiobis-(2-isopropyl-4-methylpyrimidine); 4-ethoxy-2-isopropyl-6-methylpyrimidine [72799-31-6]; 4-thioethoxy-2-isopropyl-6-methylpyrimidine; triethylphosphorothionate and triethylphosphorothiolate. Also found were several potent acetylcholinesterase inhibiters: monothionotetraethylpyrophosphate; dithionotetraethylpyrophosphate [3689-24-5]; tetraethylpyrophosphate. Model decomposition studies verified the formation of these compounds. These results were then used to identify compounds in two other samples.

Introduction

Diazinon (Figure 1) is an organophosphorothioate pesticide known chemically as 0,0-diethyl-0-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate, or by its Chemical Abstracts name and number, respectively, 0,0-diethyl 0-[6-methyl-2-(1-methyethyl)-4-pyrimidinyl] phosphorothioate [333-41-5]. It may be encountered as a wettable powder, emulsifiable concentrate, dust, paste, granular or mixed formulation under such varied commercial names as: "Basudin," "Diazitol," "Diazol," "Diazajet," "Diazide," "Dazzle," "Neocidol," "Nucidol," "G-24,480," "Dimpylate," "Gardentox," "Spectracide," "Ferti-Lome," "Alfa-Tox," "Sarolex," "Srolex," "2-Way," "3-Way," and "Agrox." Diazinon is used for the control of soil insects such as cutworms, wireworms, and maggots. It is also effective against many pests of fruits, vegetables, tobacco, field crops, pasture, grasslands and ornamentals. It is also used as a veterinary drug for the control of ticks on cattle, and blowflies and mites on sheep. Furthermore, it is used extensively in controlling cockroaches and many other household insects, grubs and nematodes in turf, seed treatment and fly control. Diazinon's synthesis, properties, metabolic fate, and animal toxicity have been reported in the literature (1-14).

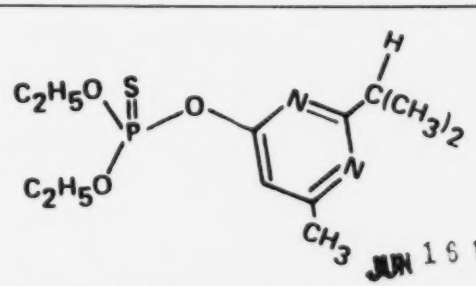

Figure 1. The chemical structure of diazinon.

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Poklis *et al.* (15) recently reported that 22 grams of diazinon were ingested in a fatal human poisoning. These authors indicated that: "The many, varied uses of diazinon, particularly around human dwellings, indicates its extensive availability and potential for accidental and intentional human exposure."

It is the purpose of this paper to relate instances where samples, eventually shown to be derived from diazinon, and believed by the parties involved to have been responsible for human poisoning, when on reaching the stage of laboratory examination and possibly when originally ingested, were a complex mixture of decomposition products of diazinon. Such samples pose problems for chemical identification, quantitative analysis, and chemical toxicity. The first part of this paper involves the initial recognition of totally decomposed diazinon from its tranformation products. Additional studies indicate the reasonableness of the transformations and possible reasons why the mixtures may be more toxic than the original diazinon.

A request for assistance, in a human poisoning, by an unknown substance, was received from a hospital in North Carolina. The following information was provided to the laboratory for this true unknown (16):

> "I am sending you under separate cover approximately 1 cc of yellow fluid which is believed to be an organophosphate liquid. This liquid was in a whiskey bottle from which Mr. [ ] drank. He had the clinical and laboratory findings of acute organophosphate poisoning. The attending physician, [ ], is interested in identifying the liquid. [ ] I would be greatly indebted to you if you would undertake the identification of this liquid."

The sample from the whiskey bottle was promptly received and stored in a refrigerator. No tissue samples were sent. The sample was a yellow liquid and would deposit white crystals. Chemical analysis was performed as quickly as possible. However, it was impossible to totally eliminate the possibility that the sample had undergone decomposition between the time of ingestion and the time the laboratory received it for examination. More probably, however, this sample had already undergone decompostion while stored in the whiskey bottle, before being ingested. The main emphasis of this paper will be on the qualitative identification of the components of this sample, since it is essential that we first know what compounds are present before we can quantitate them. With this knowledge, it is hoped that it will be easier to recognize human or environmental exposure to diazinon, even when the original organophosphorus pesticide has undergone virtually complete decomposition. In principle, standards of the compounds recognized in this study may be obtained, in the future, for quantitative measurement of tissue and environmental levels.

Experimental

Materials and Reagents

Standards of 2-isopropyl-4-methyl-6-hydroxypyrimidine, or 6-methyl-2-(1-methyethyl)-4(1H)pyrimidinone and 2-isopropyl-4-methyl-6-mercaptopyrimidine, or 6-methyl-2(1-methylethyl)-4(1H)pyrimidinothione were furnished by CIBA-GEIGY Corporation of Greensboro, North Carolina. Standards of triethylphosphorothionate and triethylphosphorothiolate were purchased from Vero Beach Laboratories, Vero Beach, Florida. The N-alkyl-N'-nitro-N-nitroso-guanidines were obtained from Aldrich Chemical Co., Milwaukee, Wisconsin. Diethylphosphoric acid, potassium diethylphosphorothioate, and dithionotetraethylpyrophosphate (Sulfotepp) standards were available from the EPA Repository in Research Triangle Park, North Carolina.

Procedures (17)

Diazoalkanes: Potassium hydroxide (2.3 g) was dissolved in 2.3 mL of water in a 125 mL Erlenmeyer flask. This was placed in a freezer until cool. Diethylether (25 mL, stabilized with 2% EtOH) was added and the mixture returned to an explosion-proof freezer for 15 minutes. Then 0.01 moles of the appropriate nitrosoguanidine (1.6 g N-ethyl-N'-nitro-N-nitrosoguanidine; 2.0 g N-amyl-N'-nitro-N-nitrosoguanidine) were weighed out. This was added cautiously to the aqueous base in the Erlenmeyer flask in several increments with swirling after each addition. When reaction subsided, the layers were allowed to separate and the upper layer was decanted into a screw-capped vial. This was then stored in a freezer.

Preparation of Standards

A few milligrams of the alkyl phosphate standards (diethylphosphoric acid, potassium diethylphosphorothioate) were weighed into a 45 mL centrifuge tube. For compounds existing as salts, a drop or two of 6 *N* HCl was added to convert the salt to the free acid. Diazopentane was added dropwise until a yellow color persisted. The solution was allowed to stand for one hour and excess diazopentane was removed by adding 0.5 mL of 0.1 *M* formic acid to the solution, followed by 5 mL of water. The unknown mixture was extracted twice with 5 mL volumes of hexane. The combined hexane extracts were evaporated to about 1 mL. This afforded standards and samples for GC analysis.

Ethylation of 2-isopropyl-4-methyl-6-hydroxypyrimidine and 2-isopropyl-4-methyl-6-mercaptopyrimidine: A few milligrams of each compound were weighed into a 45 mL centrifuge tube. Diazoethane was added dropwise until a yellow color persisted. The solution was allowed to stand for one hour, then excess diazoethane was removed by bubbling nitrogen through the solution.

Synthesis of 6,6'-dithiobis-(2-isopropyl-4-methylpyrimidine): A few milligrams of 2-isoproyl-4-methyl-6-mercaptopyrimidine were oxidatively coupled to the symmetrical disulfide by using the established literature procedure of treatment with hydrogen peroxide (18).

Diazinon conversions: Samples of diazinon were cleaned by silica column chromatography, outgassed on a vacuum line, sealed off, and submitted to thermal decomposition at 140°C for eight hours. Other samples were subjected to decomposition at 80°, 100°, and 140°C in the laboratory atmosphere for periods ranging from several minutes to several days.

Gas Chromatography

Gas chromatographic (GC) analyses were carried out on a Tracor Model 222 gas chromatograph. Two types of detectors were used. A Flame Photometric Detector (FPD) equipped with a Spectrum 1020 noise filter and a variable voltage power supply (Power Designs, Westbury, New York) was used to detect phosphorus or sulfur. A Tracor MT-222 gas chromatograph equipped with a Tracor Model 702 N-P

(nitrogen-phosphorus) detector was also used in the analysis. The operating parameters of the N-P detector were: hydrogen, 2.5 mL/min; source power, 550 v; polarizing voltage, 20 v. GC parameters for identification of the alkyl phosphate and thiophosphate acids as their pentyl derivatives were identical to those in the reference manual (17).

GC screening for the organophosphorus pesticides was based on the known relative retention times on three chromatographic columns (17): 5% OV-210 on Gas-Chrom Q 80/100 mesh; 4% SE-30/6% OV-210 on Gas-Chrom Q 80/100 mesh; and 1.5% OV-17/1.95% OV-210 on Gas-Chrom Q 80/100 mesh. All columns were operated at 200°C with 40 mL/min N_2 carrier gas. The inlet temperature was 225°C.

Mass Spectrometry

Aliquots of the intial sample (1-15 μg) were subjected to mass spectrometric analysis utilizing a Varian MAT 311A (Varian Associates, Bremen, West Germany) mass spectrometer (MS) equipped with a combination field desorption/field ionization/electron impact (FD/FI/EI) ion source. The MS was interfaced with a Model 2700 gas chromatograph. The parameters used in FD/FI analyses were ion source temperature 70°C; acceleration voltage 3 kV; extraction plate voltage 7 kV; FD emitter heating current 2-10 mA; resolution 1000. The GC/FI/FD/MS parameters were: source temperature 170°C; acceleration voltage 3 kV; extraction plate voltage 6.5 kV. The emitters were 10 micron diameter tungsten wire that had been activated in benzonitrile at a partial pressure of about 0.13 Pa under the influence of high field strength and high temperature. The microneedle length was 15-25 μm. The experimental GC/EI/MS low and high resolution parameters were: ion source temperature 225°C; acceleration voltage 3 kV; electron energy 70 eV; electron emission 2 mA; MS resolution 1000 to 9000; slit separator and connnecting lines 250°C. The 1 m x 2 mm i.d. GC glass column contained 4% Dexsil 400 on 80/100 mesh chromosorb W HP; helium flow rate 15 mL/min; injection port temperature 250°C; GC column temperature was programmed from 70°C to 250°C at 6°C/min. The mass spectra were recorded on a light beam oscillograph. Chemical ionization (CI) mass spectra were obtained on a Finnigan 3200 mass spectrometer (Finnigan Instruments, Sunnyvale, California) with a Model 6002 data system and a Model 9500 gas chromatograph. A 183 cm x 2 mm i.d. glass column packed with 1.5% OV-17/1.95% OV-210 on 80/100 mesh Gas-Chrom Q was used. A CI run was made with methane as the reagent gas, maintained at 130 Pa in the ion source operated at 60°C. Continuing studies were made using GC/low resolution MS (GC/LRMS) on a Hewlett Packard 5930A mass spectrometer (Hewlett-Packard, Palo Alto, California) equipped with a Model 5700A gas chromatograph and Model 5933A data system. Standard 70 eV conditions were used. The GC was programmed from 80°C (2 min) to 240°C at 8°C/min. GC column length and packing were the same as for the Finnigan.

Results and Discussion

In response to the request for the analysis of a sample believed responsible for a human poisoning, a detailed study was undertaken to determine the chemical nature of the unknown. The sample was submitted to an initial screening by GC using phosphorus specific detectors (17,19,20). However, no intact pesticide was identified. The sample was found to be extensively hydrolyzed to free diethylphosphoric and diethylphosphorothioic acids. These were identified by the respective GC retention times of their pentyl derivatives on a 5% OV-210 column using phosphorus and sulfur flame photometric detection (17). The suspected toxicity of the sample, combined with the inability of established routine screening methods to reveal its chemical nature, led to an increased concern for accurate characterization.

Due to the unknown nature of the toxic substance, a decision was made to apply mass spectrometric techniques to the determination of the chemical identity of the sample. Number, approximate relative concentration, and mass of molecular ions were determined by low energy ionization techniques (21,22): field ionization (FI), field desorption (FD), and chemical ionization (CI). The elemental compositions of the molecular ions and of the fragments in the electron impact ionization (EI) spectra were determined by exact mass measurements made at high resolution (HRMS). Plausible structures were assigned, based on comparison with library spectra and with respect to all available chemical evidence and sample history. Where possible, proof of structure was provided by synthesis and by identity of GC retention times and mass spectra.

Field ionization mass spectrometry, from the direct insertion probe, was initially used in order to obtain molecular ion information and to deal with potential problems of thermal decompostion and/or lack of volatility of the sample components. A representative probe field ionization spectrum is shown in Figure 2. The following molecular ions and relative abundances were determined: m/z 152 (68%), m/z 168 (3%), m/z 302 (11%) and m/z 334 (17%). These values served to give an idea of the relative proportions of the abundant components of lower volatility in the initally received sample. The true percentages would be less if the more volatile components of the sample were included.

Also, these results were not corrected for differences in response factors due to differences in ionization efficiencies or to different responses at different masses. This was due to the fact that standards were not initially available, since the chemical identity of the compounds was not known in the early stages of the analysis.

Field desorption runs also detected molecular ions at m/z 180, 196, and 198. All of these molecular ions were also found in the parallel GC/CI/MS study. Comparison of these results with electron impact spectra obtained by GC/MS confirmed the thermal and electron impact stability of the compounds in the mixture. Furthermore, GC/MS permitted the analysis of the lower level and more volatile compounds in the sample.

A problem of non-uniqueness in assignment of compositions was encountered for the experimental exact mass values determined at high resolution and shown in Table I. This problem was due to experimental error and the large number of compositions generated from the many elements considered in dealing with a true unknown. Unambiguous assignment was made, however, when certain lower mass fragments in the electron impact spectra of components of the poisoning sample, shown in Figure 3, were compared to the fragments in the mass spectrum (23) of the pesticide diazinon (structure A in Figure 4). For example, an exact mass of m/z 135.0933 fit for $C_8H_{11}N_2$ (Theory, 135.0922) and corresponded in these products to the analogous fragment

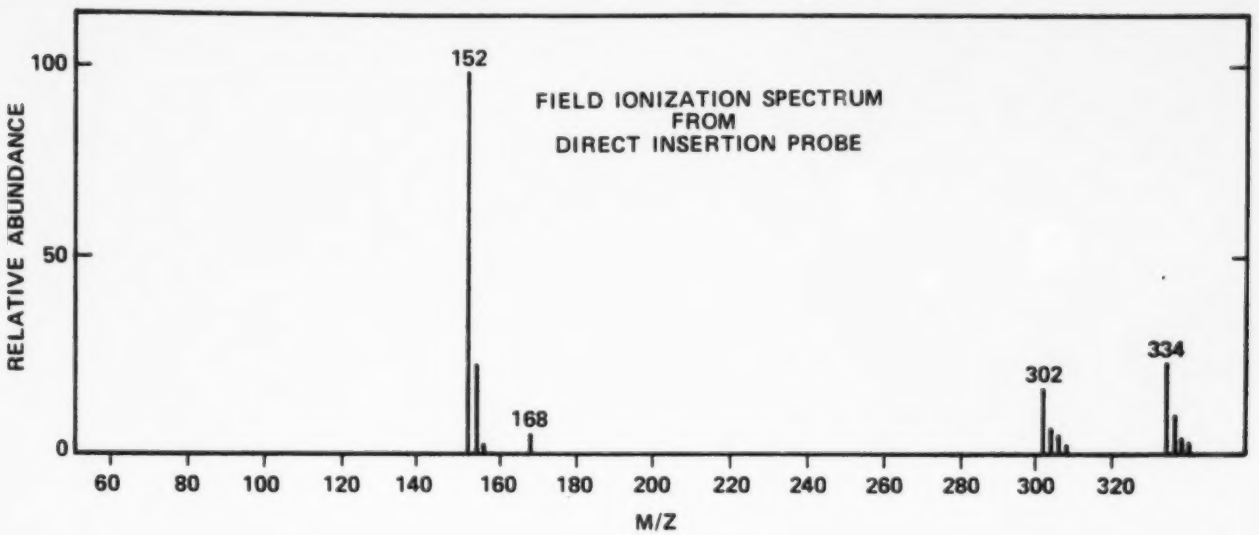

Figure 2. Field ionization spectrum of the sample mixture obtained from the direct insertion probe.

Table I. Selected Mass Spectral Data of Diazinon Transformation Products

Observed m/z	Error (ppm)	Assigned Composition	Assignments Ion	Structure	Confirmed by GC/MS of Standard
152.0938	(7.6)	$C_8H_{12}N_2O$	$M^{+\cdot}$	HOAr*	+
137.0714	(0.9)	$C_7H_9N_2O$	$M^{+\cdot}$-CH_3		
180.1254	(5.0)	$C_{10}H_{16}N_2O$	$M^{+\cdot}$	C_2H_5OAr	+
137.0707	(6.0)	$C_7H_9N_2O$	Fragment		
168 (low resolution data)			$M^{+\cdot}$	HSAr	+
196.1031	(2.0)	$C_{10}H_{16}N_2S$	$M^{+\cdot}$	C_2H_5SAr	+
163.1223	(7.9)	$C_{10}H_{15}N_2$	$M^{+\cdot}$-HS$^{\cdot}$		
198.0482	(1.0)	$C_6H_{15}O_3SP$	$M^{+\cdot}$	$(C_2H_5O)_3P=S$	+
170.0163	(2.3)	$C_4H_{11}O_3SP$	$M^{+\cdot}$-C_2H_4		
302.1554	(4.0)	$C_{16}H_{22}N_4S$	$M^{+\cdot}$	Ar-S-Ar	
287.1326	(1.7)	$C_{15}H_{19}N_4S$	$M^{+\cdot}$-CH_3.		
269.1745	(8.2)	$C_{16}H_{21}N_4$	$M^{+\cdot}$-HS$^{\cdot}$		
167.0644	(6.0)	$C_8H_{11}N_2S$	$^+$SAr		
135.0933	(8.1)	$C_8H_{11}N_2$	$^+$Ar		
334.1289	(0.6)	$C_{16}H_{22}N_4S_2$	$M^{+\cdot}$	Ar-S-S-Ar	+
335.1317	(0.9)	$C_{15}{}^{13}CH_{22}N_4S_2$	$M^{+\cdot}+1$		
336.1257	(3.6)	$C_{16}H_{22}N_4{}^{32}S^{34}S$	$M^{+\cdot}+2$		
306.04680	(4.1)	$C_8H_{20}O_6P_2S$	$M^{+\cdot}$	$(C_2H_5O)_2\overset{S}{\overset{\|}{P}}\text{-O-}\overset{O}{\overset{\|}{P}}(OC_2H_5)_2$	
322 (low resolution data)			$M^{+\cdot}$	$(C_2H_5O)_2\overset{S}{\overset{\|}{P}}\text{-O-}\overset{S}{\overset{\|}{P}}(OC_2H_5)_2$	+

*Ar is the 6-methyl-2-(1-methylethyl)-4-pyrimidinyl ring, shown in Figure 4.

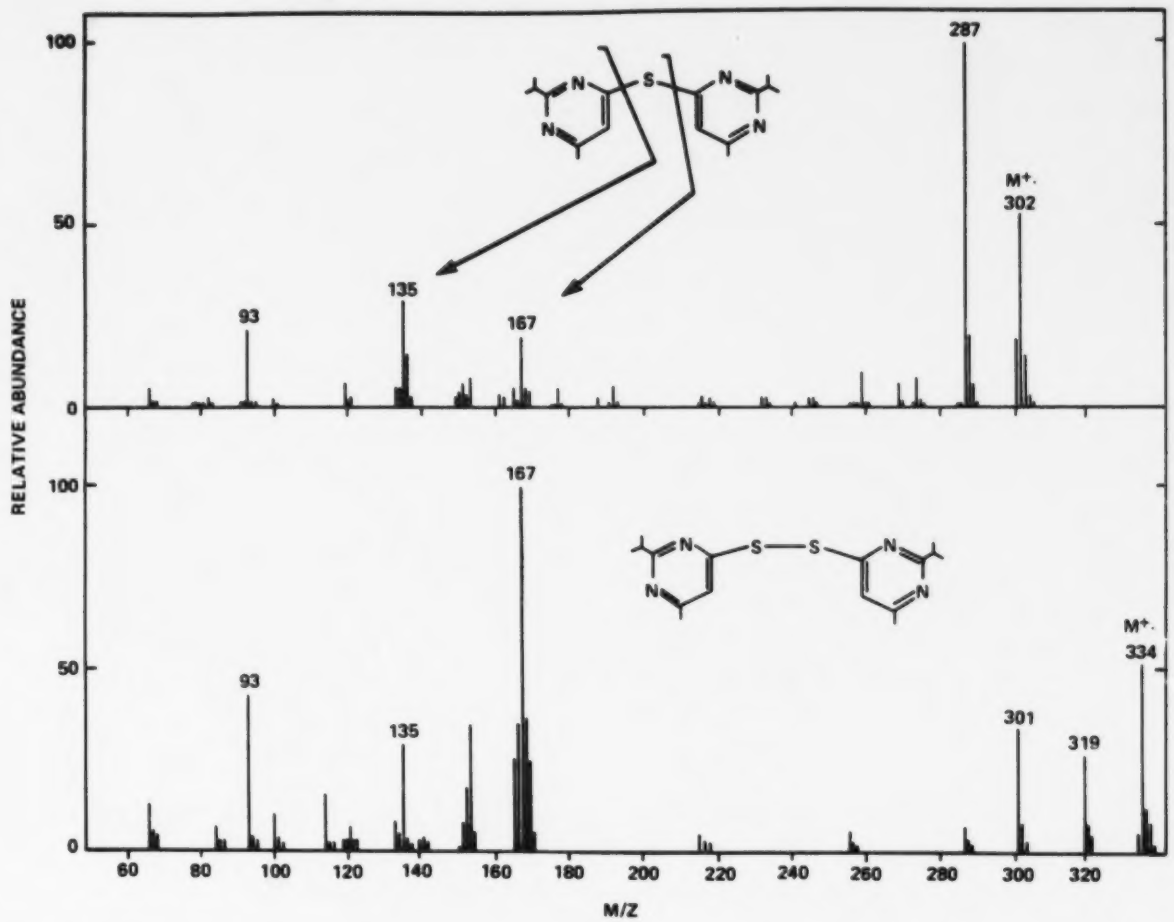

Figure 3. Electron impact mass spectra and structural assignments of two distinctive components of the sample mixture, separated by GC/MS.

formed from cleavage alpha to the oxygen in the parent diazinon; 167.0644 agreed with the addition of a sulfur atom to this fragment, for a composition of $C_6H_{11}N_2S$ (Theory, 167.0643). Furthermore, 152.0938 fit for the composition of the pyridinol fragment from diazinon (Theory, 152.0949). This allowed assignment of the m/z 152 molecular ion compound as 2-isopropyl-4-methyl-6-hydroxypyrimidine, or 6-methyl-2-(1-methylethyl)-4(1H)pyrimidinone (Structure C, Figure 4). A spectral match for this compound was obtained from the NIH/EPA Library (24). Once the 6-methyl-2-(1-methylethyl)-4-pyrimidinyl ring (Ar) was established, assignments were readily made for many of the other compounds as indicated in Table I and confirmed by synthesis and identity of mass spectrum and GC retention time. The relative retention times to aldrin, internal standard of the most abundant compounds in the sample for a GC temperature program of 80°C (two min) programmed to 240°C at the rate of 8°C/min on a 1.5% OV-17/1.95% OV-210 GC column were: alkyl benzenes (0.11-0.19), C_2H_5OAr (0.32), $(C_2H_5O)_2P=S$ (0.35), $(C_2H_5O)_2P=O(SC_2H_5)$ (0.48), C_2H_5SAr (0.53), HOAr (0.75), ArSAr (1.14), Ar-S-S-Ar (1.23).

The assigned structures of diazinon transformation products are summarized in Figure 4 and were related by a rational sequence of chemical transformations. Simple hydrolysis of diazinon (A) accounted for the formation of the diethylphosphorothioic acid (B). This, in turn, probably catalyzed the further decomposition of the starting material. Diazinon is reported to be unstable in acids and to have a half life of only 12 hours at a pH of 3.1 (25). The other product of the initial hydrolysis was the 2-isopropyl-4-methyl-6-hydroxypyrimidine (C) with a molecular ion ($M^{+\cdot}$) of m/z 152. The second type of reaction encountered was alkylation, presumably by the ethyl groups of the original ester (25). Such alkylation reactions may have accounted for the formation of the ethylether (D) and for subsequent ethylation of other compounds in the reaction scheme.

The thiono-thiolo rearrangement, the conversion of $R_1R_2P=S(OR_3)$ to $R_1R_2P=O(SR_3)$ is known to accompany the process of alkylation (26). For example, alkylation of the anion of B at the sulfur produces triethylphosphorothiolate (E). One known diazinon rearrangement product, the S-ethyl diazinon (27), was not detected in this mixture. However, the number of products which resulted with sulfur next to the aromatic ring strongly suggested that S-Ar diazinon (F in parentheses) was formed, as an intermediate. This product was not detected in the mixture, presumably, due to inherent

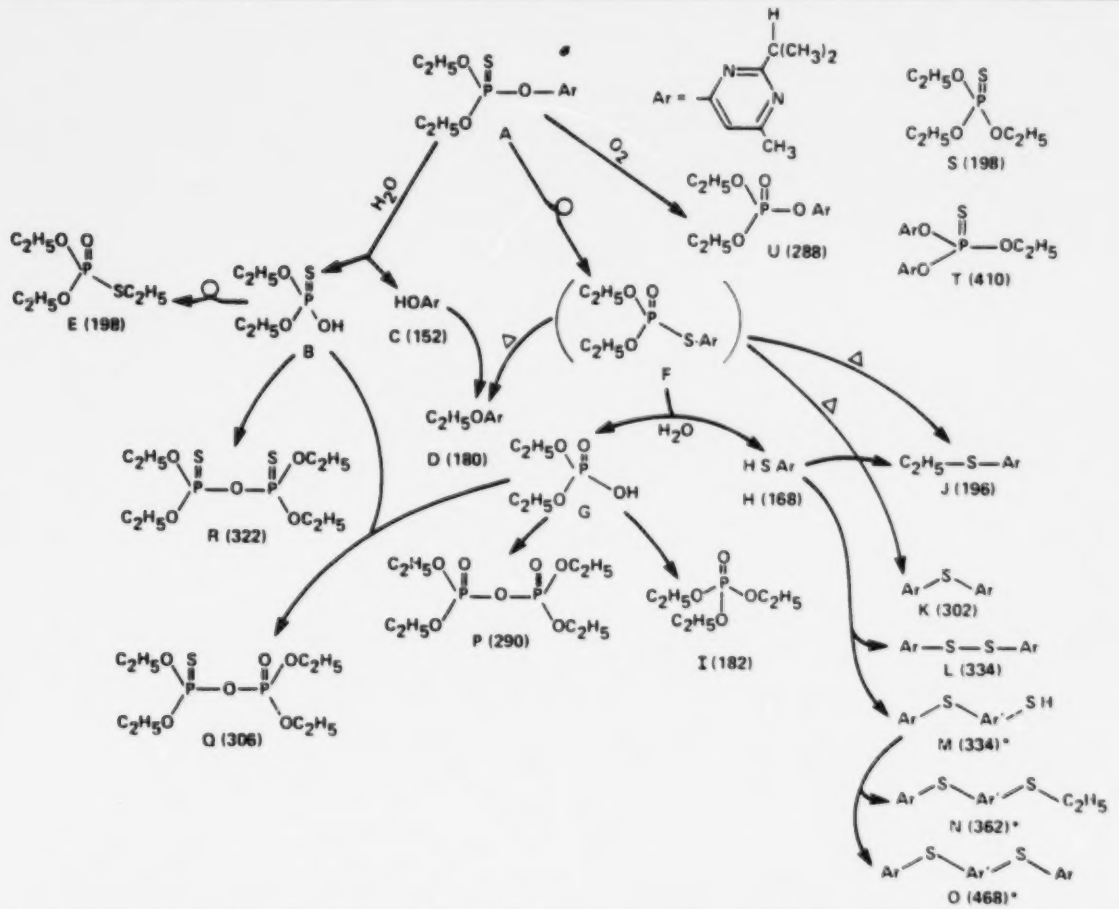

Figure 4. Reaction pathways and structural assignments of diazinon (A) transformation products found in the poisoning samples. The numbers shown in parenthesis are the masses of the experimentally determined molecular ions. Ar' means ring position was not established. See text for basis of assignment.

thermal instability and rapid hydrolysis to yield diethylphosphoric acid (G) and 2-isopropyl-4-methyl-6-mercaptopyrimidine (H, ArSH) with m/z 168. These hydrolysis products were partially ethylated, probably by remaining diazinon, to form triethylphosphate (I), m/z 182, and the thioether (J) of m/z 196. However, model thermal (140°C) diazinon decompositions carried out under *dry*, oxygen-free conditions showed the direct formation of large quantities of C_2H_5SAr (J) without the necessity of preliminary hydrolysis. The formation of analogous compounds had been observed previously in cases of the thermal decomposition of O,O-dialkyl S-aryl thiophosphates by an internal alkylation-decomposition mechanism (25,26). This further strengthened the argument in favor of the intermediate (F). Model decompositions also showed the direct formation of a large quantity of the diagnostically useful sulfide, Ar-S-Ar (K) of m/z 302. This could best be explained as an intermolecular transfer of the Ar group, probably from diazinon, to the -S-Ar group of F, with subsequent decomposition. These model experiments also showed the direct formation of C_2H_5OAr (D) under non-hydrolytic conditions, presumable by internal alkylation-decomposition of diazinon itself. The hydrolysis route may, however, remain important for lower temperature and moist environmental transformations. The 2-isopropyl-4-methyl-6-mercaptopyrimidine was shown to be susceptible to coupling by oxygen to yield the symmetrical disulfide (L) of m/z 334. Both K and L were abundant (see Figure 2) and late GC eluting compounds. The distinctive mass spectra they yielded are shown in Figure 3. The sulfide (K) and disulfide (L) proved to be "marker compounds" in the recognition of diazinon exposure in other instances.

Additional compounds (M,N,O) (Figure 4), shown with an asterisk, did not have their structures established by synthesis, but were expected to have structures similar to those produced in the coupling of substituted phenoxy radicals (28). The symbol (Ar') in Figure 3 was used to represent a disubstituted and perhaps rearranged ring structure. Evidence in favor of these species was: (1) the internal consistency of the mass shift for this series of products, (2) the occurrence of two peaks in the selected ion current profiles of m/z 334, and (3) the apparent increase of the m/z 362 signal upon treatment of the mixture with diazoethane. The m/z 468 compound was discovered in a combined liquid chromatography/mass spectrometry (LC/MS) study of diazinon which was in a storage drum and was included to complete the series. Details of this study will be given elsewhere.

The remaining series of transformation products (P,Q,R) resulted from the acid catalyzed condensation, in the presence of limited amounts of water, of diethylphosphoric and diethylphosphorothioic acids to yield tetraethylpyrophosphate

and mono- and dithionotetraethylpyrophosphates with molecular ions of m/z 290, 306, and 322, respectively.

Margot and Gysin (29,30) gave an extensive study in 1957 of the decomposition products of diazinon. These authors reported that the toxicity of diazinon decomposition mixtures was fully accounted for by the presence of the monothionotetraethylpryophosphate of m/z 306 (Q), which was first prepared by Arbusov (31). The highly toxic (32) tetraethylpyrophosphate of m/z 290 (P) is rapidly hydrolyzed while the dithionotetraethylpyrophosphate (R) of m/z 322 is relatively stable, though less toxic than Q or P. The monothionotetraethylpyrophosphate is a potent acetylcholinesterase inhibitor (29) with i_{50} values for 50% inhibition of pseudocholinesterase of 0.8 mg% for diazinon and 5.5 x 10^{-5} mg% for the monothionotetraethylpyrophosphate, a factor of 14,000 increase over diazinon itself. The more hydrolytically stable and, therefore, more prevalent dithionotetraethylpyrophosphate (R) is about a factor of 0.07, as active as the monothionopyrophosphate, still a relative acetylcholinesterase inhibitor activity of 1000 times that of diazinon. The increased potential for inhibition of acetylcholinesterase by formation of even low levels of these thionopyrophosphate esters could make the solutions of decomposed diazinon quite poisonous. For example, even a 0.1% by weight solution of the monothiono compound (Q) would still be fourteen times more inhibitory of cholinesterase activity than diazinon itself. A 1.4% solution of (R) would have a comparable activity.

Additional compounds found in the sample were a small amount of the oxone of diazinon (U), presumable due to air oxidation, or condensation of (C) and (G). Compounds S and T are known to be contaminants present in commercial diazinon due to the method of preparation (30).

There are some differences in our findings and the report of Margot and Gysin in that they found the 2-isopropyl-4-methyl-6-hydroxypyrimidine (C) in only 0.5%, while in our study it was the main component. It seems reasonable that differences in temperature and hydrolysis conditions can greatly influence the product distribution. These authors found a disubstituted product $(ArO)_2(C_2H_5O)P=O$, but neither the symmetrical disulfide of m/z 334 (L) nor its precursor the 168 m/z species, the mercaptopyrimidinol (H), were reported in their reaction mixture. Also, a number of minor species were further recognized in the GC/LRMS study of the sample. Some of these compounds were related to traces of carrier solvent, or impurities. These species are listed in Table II.

A recent study of diazinon decomposition on a common absorbent for pesticidal compounds (33) has reported products resulting from the exchange of the propyl group. This may offer an explanation for the m/z 212 species present in Table II.

After the completion of the initial study of the decomposed diazinon sample, our laboratory encountered two other occurrences of this. A sample, in a sealed scintillation vial, was delivered from Egypt. It was stated that this sample was suspected of having been involved in a human poisoning there. Upon receipt by our laboratory, the sample was found to be a yellow, crystalline solid, quite in contrast to the yellow solutions of diazinon in aromatic hydrocarbon solvents (see Table II) which we had encountered. Because of our earlier study, we were able to recognize in this sample most of the compounds shown in Figure 4, specifically, those designated as J, E, S, C, R, K, L, and I. However, only the dithionotetraethylpyrophosphate was recognized of the P, Q, R series. The free acids were not analyzed. Again, no intact diazinon could be detected by GC/MS of this sample. Unfortunately, it is not possible to exclude decompostion of the sample after the alleged poisoning episode occurred, although an effort was made to preserve the sample by refrigeration, before analysis. Whenever decomposition occurred, the analytical problem of recognition would remain.

Table II. Additional Apparent Molecular Ions (by GC/LRMS) and Possible Structural Assignments for the Remaining Components in the Sample

Observed $M^+\cdot$ (m/z)	Assignment	Possible Origin
106	C_2-alkylbenzene	Solvent residue
120	C_3-alkylbenzene	Solvent residue
134	C_4-alkylbenzene	Solvent residue
166	CH_3OAr (?)	
168		
182	$(C_2H_5O)_3P=O$	See Text
212	$(C_3H_7O)(C_2H_5O)_2P=S$	See Text
220	di-tert-butylhydroquinone (24)	Contamination
242		
248	di-tert-amyl-p-benzoquinone (24)	Contamination
270		
284		
288	diazoxon	See Text
290	tetraethylpyrophosphate	See Text
298		
308		
314		
324		
326		
346		
356		
410	$(ArO)_2\overset{S}{\overset{\|}{P}}-OC_2H_5$	From synthesis

The marker compounds designated as K, L, J, D, C, O, and S were also found in a sample of pesticide, which proved to be diazinon, contained within an unlabelled storage drum. Thus, recognition of these compounds could prove helpful in the characterization of materials which environmental toxicologists may encounter at old pesticide disposal sites.

Studies on the control analytical standard diazinon showed that it contained detectable quantities of compounds designated in Figure 4 as D, S, H, R, and T. This is indicative of the normal level of decomposition and/or contamination by impurities from manufacture of most diazinon samples. Silica column chromatography was found to eliminate most impurities.

Conclusion

Diazinon may be encountered in forms other than the intact pesticide. In the presence of moisture and heat, diazinon may decompose into a complex mixture of chemical compounds which can make recognition by the usual analytical methods used to detect organophosphorus pesticides impossible. If sample decomposition occurs after human or environmental exposure, but before submission of a sample to analysis, then the analytical recognition problem will be encountered. Should the diazinon decompose before human or

environmental exposure, samples may contain potent acetylcholinesterase inhibitors as well as being difficult to identify. Analysis of three decomposed samples by mass spectrometric techniques, followed by model decomposition experiments, established that several chemical compounds will be formed from the decomposition reaction and may be used as chemical markers of diazinon exposure. Qualitative identification of these compounds was necessary so that the appropriate analytical standards could be made available.

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